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# Effects of water stress on growth phenology photosynthesis and leaf water potential in *Stipagrostis ciliata* (Desf.) De Winter in North Africa

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**Abstract:** *Stipagrostis ciliata* (Desf.) De Winter is a pastoral  $C_4$  grass grown in arid regions. This research work focused on assessing the growth of *S. ciliata* accessions derived from two different climate regions (a wet arid region in the Bou Hedma National Park in the central and southern part of Tunisia (coded as WA), and a dry arid region from the Matmata Mountain in the south of Tunisia (coded as DA)) under water stress conditions. Specifically, the study aimed to investigate the phenological and physiological responses of potted *S. ciliata* seedlings under different water treatments:  $T_1$  (200 mm/a),  $T_2$  (150 mm/a),  $T_3$  (100 mm/a) and  $T_4$  (50 mm/a). Growth phenology, net photosynthesis ( $P_n$ ), stomatal conductance ( $g_s$ ), midday leaf water potential ( $\Psi_{md}$ ), predawn leaf water potential ( $\Psi_{pd}$ ), soil water content (SWC) and soil water potential ( $\Psi_s$ ) were observed during the water stress cycle (from December 2016 to November 2017). The obtained results showed that the highest growth potential of the two accessions (WA and DA) was recorded under treatment  $T_1$ . The two accessions responded differently and significantly to water stress. Photosynthetic parameters, such as  $P_n$  and  $g_s$ , decreased sharply under treatments  $T_2$ ,  $T_3$  and  $T_4$  compared to treatment  $T_1$ . The higher water stress increased the R/S ratio (the ratio of root dry biomass to shoot dry biomass), with values of 1.29 and 2.74 under treatment  $T_4$  for accessions WA and DA, respectively. Principal component analysis (PCA) was applied, and the separation of *S. ciliata* accessions on the first two axes of PCA (PC1 and PC2) suggested that accession DA was detected in the negative extremity of PC1 and PC2 under treatments  $T_1$  and  $T_2$ . This accession was characterized by a high number of spikes. For treatments  $T_3$  and  $T_4$ , both accessions were detected in the negative extremity of PC1 and PC2. They were characterized by a high root dry biomass. Therefore, *S. ciliata* accessions responded to water stress by displaying significant changes in their behaviours. Accession WA from the Bou Hedma National Park (wet arid region) showed higher drought tolerance than accession DA from the Matmata Mountain (dry arid region). *S. ciliata* exhibits a significant adaptation capacity for water limitation and may be an important species for ecosystem restoration.

**Keywords:** *Stipagrostis ciliata*; drought stress; water deficit; gas exchange; arid regions; Tunisia

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## 1 Introduction

Water stress is among the most important and devastating phenomena in the world (Fahad et al.,

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2017) and its frequency is likely to increase because of climate change (Batool et al., 2020; Adaawen, 2021; Roy et al., 2021). In fact, in agreement with IPCC (2014), climate change in arid regions will decrease water resources and will consequently affect the productivity of biological ecosystems. From this perspective, the decrease in soil water is becoming an important field of research aiming to explore the response of plants to water deficit to improve agricultural management practices and predict the fate of natural vegetation (Raza et al., 2019). On the other side, it has been reported that the increase of CO<sub>2</sub> rate is driving the climate in the Mediterranean and tropical areas towards important changes, mainly represented by high temperatures and low annual precipitation (Santos et al., 2014). Plant growth and productivity are adversely affected by water stress. However, morphological, metabolic and physiological modifications have appeared in all plant organs (Ma et al., 2020). Drought also decreased leaf areas, owing to the loss of turgor and reduction of leaf numbers (Farooq et al., 2010). Shao et al. (2008) proved that drought also suppresses leaf expansion and tillering as well as leaf area reduction due to early senescence. Wellstein et al. (2017) highlighted that drought can generate a series of reductions in morphological and physiological traits, such as plant height, specific leaf area, leaf water potential, leaf tissue density and root length. Then, these variations of plant traits may lead to a decline in yield and nutritive value. Within this framework, Seleiman et al. (2019) emphasized that water stress is a major environmental and multidimensional stress factor leading to reduced crop yield and biomass productivity worldwide. Water stress severely affects plant growth and generates a strong relapse in reproduction.

The restoration of degraded ecosystems (rangelands and grasslands) under these environmental conditions becomes a necessity to maintain biological productivity. The choice of a plant candidate species for restoration is a crucial issue (Derbel et al., 2009; Aronson et al., 2020). For instance, to choose plant species adapted to the Mediterranean climate conditions, it is necessary to investigate the relationship of soil water status with phenological and physiological parameters of plants (Maestre et al., 2021) to improve vegetation adaptation and production against water stress. However, Jones (2007) asserted that the measurement of plant and soil water status is crucial in any experiment where one is concerned with exploring the effects water stress on plant growth. Overall, soil water potential ( $\Psi_s$ ) is an ecological parameter that affects seed germination, seedling establishment, plant nutrition and plant growth (Niu et al., 2021). West et al. (2008) clarified how plants use water resources in the soil and how this affects their growth and ability to resist the greatest droughts. Indeed, to accurately predict species dynamics and ecosystem water balances, investigating how climate change will alter plant water acquisition, belowground interactions for water resources and their subsequent impact on plant functions is highly significant. Hence, the knowledge of  $\Psi_s$  is fundamental to understanding plant water stress tolerance in arid regions. Soil moisture storage stands for the main water source for plants between precipitation events, affecting plant growth phenology (Brocca et al., 2017).

Water stress corresponds to one of the most limiting environmental parameters to plant productivity and can be triggered by both atmospheric and soil water deficits (Ding et al., 2021). In arid regions of North Africa and South Sahara, the response of C<sub>4</sub> plant photosynthesis pathways to water deficit has been scarcely addressed. Furthermore, C<sub>4</sub> grasses dominate in hot Saharan arid regions (Ouled Belgacem et al., 2006; Ghannoum, 2009). In this regard, Christin and Colin (2014) proved that the physiological advantages conferred by C<sub>4</sub> plants are important for the ecological dominance of C<sub>4</sub> plants in arid environments. Understanding the mechanism underlying the resistance of perennial species, especially grasses to drought stress, is of extreme importance for improving the productivity of perennial species in arid regions. However, for ecological restoration programmes, some species such as those in the perennial genus *Stipagrostis* (e.g., *Stipagrostis ciliata* (Desf.) De Winter, *S. obtusa* (Delile) Nees and *S. plumosa* (L.)) can be regarded as good candidate species for effective pastoral improvement under arid Saharan and tropical arid bioclimate conditions.

*S. ciliata* is a native arid species characterized by an important pastoral value in arid regions. It is a pastoral C<sub>4</sub> plant species belonging to the Poaceae family. *S. ciliata* has a wide-spread

distribution in the hotter and drier parts in arid regions (Kellogg, 2013). This perennial densely tufted grass species is up to 1 m high, with basally concentrated leaves. It forms a rounded hummock with the flowers emerging in clumps. *S. ciliata* exhibits high ecological abilities to tolerate Saharan and arid bioclimate conditions, gypsy soil and mobile sandy soil (Daur, 2012). Therefore, it is a useful grass species to fix sand in arid regions. This species plays an important role in protecting the soil from erosion and reducing water loss. In addition, *S. ciliata*, a Mediterranean arid species, can grow under precipitation greater than 250 mm/a (Daur, 2012). Considering the predictable climate changes in Saharan region, particularly in Tunisia (Ben Mariem and Chaieb, 2017), and the predictable decrease of precipitation in arid and tropical regions (IPCC, 2014), it would be promising to study the phenological and ecophysiological characteristics of *S. ciliata* and how this species reacts against conditions of water stress resulting from the possible precipitation decrease. Therefore, to answer this question, we subjected this species to artificial water stress. As far as our study is concerned, the response of this grass species to the applied experimental conditions under an arid climate will probably be the same as its response under a dry tropical climate.

From this perspective, the present study was conducted at an experimental site in the south of Tunisia. The main objectives of our investigation were (i) assessing the impact of water stress on the physiological and phenological characteristics of *S. ciliata* through analysing accessions from soil origins and (ii) evaluating the relationship between soil water content (SWC) and plant ecophysiology behaviour. The elaborated hypothesis corresponds to the fact that water stress would give rise to significant changes in terms of the ecophysiological and growth responses of *S. ciliata* accessions. The results will be crucial at the level of evaluating the variation for tolerance to water stress of this perennial grass, which may stand as potential candidate for the restoration of degraded arid rangelands as well as dry tropical grasslands.

## 2 Materials and methods

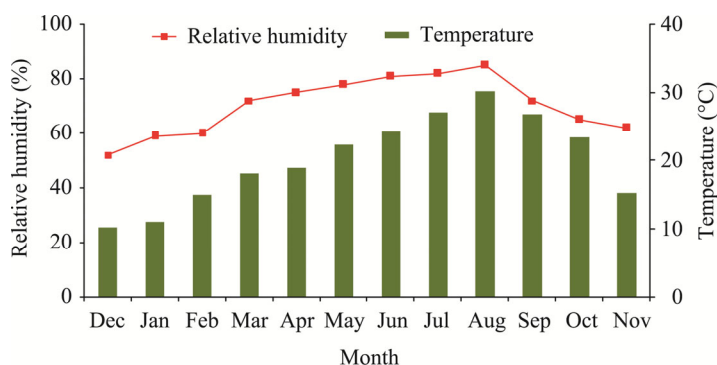
### 2.1 Plant growth and experimental design

*S. ciliata* seed accessions were collected in October 2015 in North Africa. Accessions were selected from opposing arid environments in Tunisia. The first accession was picked up from the protected Bou Hedma National Park (34°39'N, 09°48'E) located in the Governorate of Sidi Bouzid (the central and southern part of Tunisia). The climate of this region is wet arid, characterized by irregular annual precipitation (200–250 mm) and dry summer period. Soil in the protected Bou Hedma National Park belongs to the sandy soil. *S. ciliata* accession from this region was coded herein as WA. The second accession was collected from the Matmata Mountain in the south of Tunisia (33°53'N, 10°01'E). The climate of this region is dry arid, characterized by lower annual precipitation (50–150 mm) and high summer temperature. Soil in the the Matmata Mountain belongs to the loam soil. *S. ciliata* accession from this region was coded herein as DA.

The mature seeds for each accession sown in 30 pots in November 2015 were processed under a sheltered greenhouse in the experimental field localized in Sfax City (34°43'N, 10°41'E) in the central and eastern part of Tunisia. The pots were 20 L in capacity, 30 cm in diameter and 30 cm in depth. The volume of the used pots was thus 0.021 m<sup>3</sup>, which resembles the volume of the rhizosphere for the normal growth of Poaceae species. This was in good accordance with what Kharrat-Souissi et al. (2012, 2013, 2014) reported when examining the behaviour of *Cenchrus ciliaris* species, which proved to be very comparable to that of *S. ciliata*. The chemical composition of the potting soil was 6.64 mmol/L of Na, 3.16 mmol/L of K and 0.44% of CaSO<sub>4</sub>·2H<sub>2</sub>O. The total CaCO<sub>3</sub> content of the soil was about 13.00%, the organic matter percentage was 3.70% and the electric conductivity was 3.7 mS/cm. Each pot contained only one individual. Irrigation water used during the establishment year contained 1.00 g/L of NaCl. One year after sowing (i.e., November 2016), namely as soon as adult plants were obtained, they were cut above the soil surface. This procedure was conducted for each plant to simulate the zero level

of growth during the experimental period (from December 2016 to November 2017). The water treatments (water stress levels) of plants were designed as follows: T<sub>1</sub> (200 mm/a), T<sub>2</sub> (150 mm/a), T<sub>3</sub> (100 mm/a) and T<sub>4</sub> (50 mm/a). The experimental design was made with 10 replications examining water regimes at four levels of water stress (T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, and T<sub>4</sub>) for the two investigated accessions (WA and DA). In fact, according to the IPCC report (IPCC, 2014), arid Tunisia is subject to climate change over the next few decades. This change will be mainly marked by falling precipitation and rising temperatures. This climate change will affect the different types of climates in the country. We expected to predict the response of perennial grasses to this climate change by carrying out the response tests of *S. ciliata* to the amounts of future precipitation that will mark the Tunisian arid regions.

The experiments were carried out under environmental conditions with natural sunlight and temperature. Mean temperature and relative humidity were measured during the experimental period (from December 2016 to November 2017) with Combined Thermohygrograph 3.015 Wittich & Visser, UK. Mean monthly temperature varied between 11°C and 30°C, and mean relative humidity ranged between 52% and 85% (Fig. 1).



**Fig. 1** Relative humidity and mean monthly temperature during the experimental period (from December 2016 to November 2017) in a shelter greenhouse in the experimental field in the central and eastern part of Tunisia

## 2.2 Phenological and physiological parameters

Phenological observations on leaf and spike development growth were performed on every second week of each month. The following parameters were determined: tiller, spike and leaf numbers. Net photosynthesis ( $P_n$ ) and stomatal conductance ( $g_s$ ) were measured between 10:00 am (LST) and noon, biweekly on sunny days. As for the new leaves of *S. ciliata*, a Scholander Pressure Chamber (PMS Instrument Company, Oregon, USA) (Scholander et al., 1965) was used. For each water treatment and each studied accession, twenty plants were randomly chosen. Leaf water potential was specified with a Scholander Pressure Chamber (Scholander et al., 1965) following the precautions proposed by Turner (1988). For each water treatment, leaves were randomly selected from plants under both accessions for the determination of predawn leaf water potential ( $\Psi_{pd}$ ) and midday leaf water potential ( $\Psi_{md}$ ).  $\Psi_{pd}$  and  $\Psi_{md}$  were measured at 05:00 am and 14:00 pm every two weeks, respectively. According to Ritchie and Hinckley (1975), the time between excision and determination was approximately 40–50 s.

SWC was determined by a Moisture meter, Model HH2 (Delta Device, Cambridge, UK) periodically at 09:00 am every two weeks during the experimental period. SWC was considered as an index of drought intensity.  $\Psi_s$ , which is defined as the potential energy of water in the soil, was measured with a New Automated Psychrometer Scanner (Model PSYPRO, Type Vescor, Washington University, Washington, USA) placed at a 20-cm depth at 09:00 am every two weeks. The values of  $\Psi_s$  were quantified relatively to a standard state where water has no solutes, free from external forces except gravity (Hillel, 1982). To determine root dry biomass and shoot dry biomass at the end of the experiment (in November 2017), we scraped the roots carefully and

cleaned them with water. Shoots and roots were dissociated and dried at 75°C to obtain dry weight. Then, root dry biomass and shoot dry biomass were determined. The ratio of root dry biomass to shoot dry biomass was determined as the R/S ratio.

## 2.3 Statistical analyses

All statistical analyses were performed using SPSS 12.0. Design-Expert version 12 software was applied for the experimental design and statistical analyses in this research work. Differences in phenological and physiological parameters were tested using a two-way analysis of variance (ANOVA) according to Tukey's test (significance level of  $P < 0.05$ ). Moreover, the data recorded in this study were submitted to a normalized principal component analysis (PCA) (Thioulouse et al., 1997). Soil characteristics as well as physiological and phenological parameters assessed throughout the study (from different water treatments) were considered. Discrimination between the effects of different levels of drought ( $T_1$ ,  $T_2$ ,  $T_3$  and  $T_4$ ) in both accessions (WA and DA) was evaluated by observing the projection of plots of the extracted factors on the factorial plane consisting of the statistically significant axis of the PCA. A simple  $\log(x+1)$  transformation (where  $x$  is the value of variable) was applied to the PCA data to correctly stabilize variance (Frontier, 1973).

## 3 Results

### 3.1 Growth phenology and biomass of *S. ciliata*

The initiation of the growth cycle of *S. ciliata* occurred in late winter (temperature  $\geq 15^\circ\text{C}$ ) and appeared to be associated with the increase of temperature in the experimental field (Fig. 1). The results of main leaf emergence under four water treatments ( $T_1$ ,  $T_2$ ,  $T_3$  and  $T_4$ ) are illustrated in Table 1. During the study period, leaf numbers varied significantly among all water treatments for both accessions (Table 1). The leaf growth of accession WA was enhanced under treatment  $T_1$ . Indeed, the high number of leaves produced by accession WA was 28.33 ( $\pm 1.53$ ) leaves/tiller for treatment  $T_1$ , while accession DA produced only 21.00 ( $\pm 1.00$ ) leaves/tiller under the same treatment.

**Table 1** Effects of accessions and water treatments on the growth phenology and biomass of *Stipagrostis ciliata* (Desf.) De Winter

Acession	Treatment	Leaf (number)	Spike (number)	Tiller (number)	Shoot dry biomass (g/plant)	Root dry biomass (g/plant)	R/S ratio
WA	$T_1$	28.33 $\pm$ 1.53 <sup>a</sup>	21.33 $\pm$ 1.53 <sup>a</sup>	15.00 $\pm$ 1.00 <sup>a</sup>	303.33 $\pm$ 16.26 <sup>a</sup>	60.66 $\pm$ 7.06 <sup>a</sup>	0.20 $\pm$ 0.03 <sup>a</sup>
	$T_2$	23.00 $\pm$ 1.00 <sup>b</sup>	15.66 $\pm$ 2.08 <sup>b</sup>	10.00 $\pm$ 1.00 <sup>b</sup>	248.00 $\pm$ 3.00 <sup>b</sup>	66.00 $\pm$ 3.66 <sup>a</sup>	0.27 $\pm$ 0.02 <sup>a</sup>
	$T_3$	14.33 $\pm$ 1.53 <sup>c</sup>	7.00 $\pm$ 1.00 <sup>c</sup>	5.00 $\pm$ 1.00 <sup>c</sup>	125.00 $\pm$ 3.00 <sup>c</sup>	95.00 $\pm$ 3.00 <sup>b</sup>	0.76 $\pm$ 0.02 <sup>b</sup>
	$T_4$	8.17 $\pm$ 0.76 <sup>d</sup>	4.00 $\pm$ 1.00 <sup>c</sup>	4.00 $\pm$ 1.00 <sup>c</sup>	85.00 $\pm$ 4.36 <sup>d</sup>	109.00 $\pm$ 2.65 <sup>c</sup>	1.29 $\pm$ 0.07 <sup>c</sup>
DA	$T_1$	21.00 $\pm$ 1.00 <sup>a</sup>	32.00 $\pm$ 2.00 <sup>a</sup>	13.00 $\pm$ 1.00 <sup>a</sup>	182.67 $\pm$ 7.37 <sup>a</sup>	19.67 $\pm$ 2.08 <sup>a</sup>	0.11 $\pm$ 0.01 <sup>a</sup>
	$T_2$	16.00 $\pm$ 1.00 <sup>b</sup>	24.67 $\pm$ 1.53 <sup>b</sup>	7.00 $\pm$ 1.00 <sup>b</sup>	128.00 $\pm$ 3.00 <sup>b</sup>	25.00 $\pm$ 2.00 <sup>a</sup>	0.20 $\pm$ 0.02 <sup>a</sup>
	$T_3$	8.00 $\pm$ 1.00 <sup>c</sup>	7.67 $\pm$ 1.53 <sup>c</sup>	4.00 $\pm$ 1.00 <sup>c</sup>	72.67 $\pm$ 8.74 <sup>c</sup>	66.67 $\pm$ 3.79 <sup>b</sup>	0.92 $\pm$ 0.08 <sup>b</sup>
	$T_4$	4.00 $\pm$ 1.00 <sup>d</sup>	3.00 $\pm$ 1.00 <sup>d</sup>	2.00 $\pm$ 1.00 <sup>c</sup>	46.00 $\pm$ 4.58 <sup>d</sup>	125.67 $\pm$ 4.04 <sup>c</sup>	2.74 $\pm$ 0.18 <sup>c</sup>
Significance							
Acession		***	***	*	*	***	*
Acession WA $\times$ Treatment		***	***	***	***	***	***
Acession DA $\times$ Treatment		***	***	***	***	***	***

Note: The water treatments (water stress levels) of plants were designed as follows: 200 mm/a for  $T_1$ , 150 mm/a for  $T_2$ , 100 mm/a for  $T_3$  and 50 mm/a for  $T_4$ . *S. ciliata* accession from a wet arid region (Bou Hedma National Park) was coded as WA, and *S. ciliata* accession from a dry arid region (Matmata Mountain) was coded as DA. R/S ratio, ratio of root dry biomass to shoot dry biomass. \*, significant at  $P < 0.05$  level; \*\*\*, significant at  $P < 0.001$  level. Different lowercase letters in each column indicate significantly different at  $P < 0.05$  level among the four water treatments for the same accession.



The tiller production of *S. ciliata* during the growth year was the highest under treatment T<sub>1</sub>, with the values of 15.00 (±1.00) and 13.00 (±1.00) tillers/stem, respectively, for accessions WA and DA (Table 1). The tiller number of each accession responded differently to water treatment ( $P<0.06$ ). Accession WA developed more tillers per plant under all treatments, while accession DA exhibited lower numbers. The number of tillers was positively correlated with the number of leaves and shoot dry biomass under all treatments (Table 2). The reproductive intensity varied significantly between treatment and accession ( $P<0.01$ ). The data illustrated in Table 1 proved that irrigation of *S. ciliata* accessions with 200 mm/a of water (T<sub>1</sub>) presented a high spike number compared to plants irrigated with 150 mm/a (T<sub>2</sub>), 100 mm/a (T<sub>3</sub>), and 50 mm/a (T<sub>4</sub>) of water, regardless of accessions. However, the variation of the spike number depends on the treatment level. This could be assigned to the presence of significant interactions between treatment and accession for WA and DA. Nevertheless, the high number of spikes produced by each individual was 21.33 (±1.53) and 32.00 (±2.00) under treatment T<sub>1</sub>, respectively, for accessions WA and DA. The difference among the spike number was highly significant ( $P<0.001$ ) at the treatment level. The reproductive phase was earlier under treatment T<sub>1</sub> for both accessions. Spike production was enhanced under treatment T<sub>1</sub>. Water stress was the most significant variable affecting the phenological parameters measured in this study, exhibiting significant differences in all phenological parameters. Both accessions (WA and DA) responded differently and significantly to water stress, displaying different values for leaf, tiller and spike numbers (Table 1).

Root dry biomass and shoot dry biomass data as well as the R/S ratio are illustrated in Table 1. A significant effect of water treatment on the plant biomass production of *S. ciliata* was recorded. Accession DA also tended to have a low root dry biomass under treatment T<sub>1</sub>, with 19.67 (±2.08) g/plant. However, if water stress increased, shoot dry biomass decreased slowly while root dry biomass increased rapidly. Root dry biomass varied with treatment and accession with a significant interaction for the combination of accession and treatment (Table 1). The R/S ratio for both accessions increased significantly with increasing water stress levels (Table 1). Accessions WA and DA had a higher R/S ratio under treatment T<sub>4</sub>, with values of 1.29 (±0.07) and 2.74 (±0.18), respectively.

**Table 2** Pearson's correlation coefficients among morphological and physiological characteristics of *S. ciliata* accessions

Variable	Leaf	Spike	Tiller	Shoot dry biomass	Root dry biomass	R/S ratio	Total biomass	$\Psi_{md}$	$\Psi_{pd}$	$P_n$	$g_s$	SWC	$\Psi_s$
Leaf	1.000												
Spike	0.732**	1.000											
Tiller	0.923**	0.809**	1.000										
Shoot dry biomass	0.968**	0.605*	0.904**	1.000									
Root dry biomass	-0.616	-0.916	-0.647	-0.469	1.000								
R/S ratio	-0.804	-0.759	-0.745	-0.708	0.829**	1.000							
Total biomass	0.802**	0.249	0.714**	0.908**	-0.055	-0.407	1.000						
$\Psi_{md}$	-0.694	-0.713	-0.729	-0.621	0.697*	0.668*	-0.371	1.000					
$\Psi_{pd}$	-0.716	-0.749	-0.760	-0.652	0.709**	0.648*	-0.400	0.966**	1.000				
$P_n$	0.948**	0.622*	0.919**	0.975**	-0.488	-0.675	0.871**	-0.655	-0.677	1.000			
$g_s$	0.959**	0.753**	0.908**	0.931**	-0.670	-0.779	0.734**	-0.705	-0.742	0.923**	1.000		
SWC	0.922**	0.907**	0.922**	0.848**	-0.841	-0.848	0.559*	-0.797	-0.818	0.857**	0.943**	1.000	
$\Psi_s$	-0.858	-0.811	-0.819	-0.764	0.812**	0.841**	-0.477	0.835**	0.826**	-0.771	-0.902	-0.940	1.000

Note:  $\Psi_{md}$ , midday leaf water potential;  $\Psi_{pd}$ , predawn leaf water potential;  $P_n$ , net photosynthesis;  $g_s$ , stomatal conductance; SWC, soil water content;  $\Psi_s$ , soil water potential. \* and \*\* indicate significance at  $P<0.05$  and  $P<0.01$  levels, respectively.

### 3.2 Physiological parameters of *S. ciliata*

Significant effects of water stress on the  $g_s$  and  $P_n$  of both accessions were highlighted in Table 3. The highest values of  $P_n$  were observed for accessions WA and DA under treatment T<sub>1</sub> (62.4 (±2.62) and 33.17 (±0.76)  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ , respectively). The maximum photosynthetic activity coincided with the important vegetative growth period of *S. ciliata* seedlings. A significant decrease in  $P_n$  for both accessions was detected under all treatments (Table 3). This decrease in  $P_n$  was associated with the stopping growth in both accessions. Photosynthetic assimilation decreased under water stress. Accession WA grown under severe treatment T<sub>4</sub> demonstrated a decrease in  $P_n$  (83.00%) compared to treatment T<sub>1</sub>. For accession DA, the reduction of  $P_n$  was highly significant under treatments T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>. This reduction can be attributed to a reduction in  $g_s$ . There were significant differences between accessions in the gas exchange parameters (Table 3). Similar results were found regarding  $g_s$  for both accessions. A high positive correlation was found between  $g_s$  and  $P_n$ , with  $R^2=0.923$  (Table 2).

**Table 3** Effects of accessions and water treatments on the physiological parameters and  $\Psi_s$  of *S. ciliata*

Accession	Treatment	$\Psi_{md}$ (MPa)	$\Psi_{pd}$ (MPa)	$P_n$ ( $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ )	$g_s$ ( $\text{mmol}/(\text{m}^2\cdot\text{s})$ )	$\Psi_s$ (MPa)
WA	T <sub>1</sub>	-0.99±0.10 <sup>a</sup>	-0.65±0.18 <sup>a</sup>	62.40±2.62 <sup>a</sup>	244.00±8.64 <sup>a</sup>	0.63±0.02 <sup>a</sup>
	T <sub>2</sub>	-2.28±0.75 <sup>ab</sup>	-1.45±0.50 <sup>ab</sup>	40.97±1.76 <sup>b</sup>	216.33±29.10 <sup>a</sup>	1.02±0.12 <sup>b</sup>
	T <sub>3</sub>	-2.20±0.85 <sup>ab</sup>	-1.66±0.77 <sup>ab</sup>	18.07±2.00 <sup>c</sup>	130.33±5.69 <sup>b</sup>	1.39±0.11 <sup>b</sup>
	T <sub>4</sub>	-3.57±0.40 <sup>b</sup>	-2.63±0.15 <sup>b</sup>	11.60±2.62 <sup>d</sup>	66.00±5.29 <sup>c</sup>	1.14±0.14 <sup>c</sup>
DA	T <sub>1</sub>	-0.74±0.06 <sup>a</sup>	-0.51±0.10 <sup>a</sup>	33.17±0.76 <sup>a</sup>	192.67±5.00 <sup>a</sup>	1.00±0.09 <sup>a</sup>
	T <sub>2</sub>	-2.28±0.75 <sup>b</sup>	-1.45±0.51 <sup>ab</sup>	23.00±2.00 <sup>b</sup>	161.33±3.00 <sup>b</sup>	1.34±0.20 <sup>a</sup>
	T <sub>3</sub>	-2.20±0.85 <sup>ab</sup>	-1.76±0.76 <sup>b</sup>	14.22±0.77 <sup>c</sup>	114.67±5.00 <sup>c</sup>	1.41±0.45 <sup>b</sup>
	T <sub>4</sub>	-3.48±0.28 <sup>b</sup>	-2.33±0.15 <sup>b</sup>	7.17±1.04 <sup>d</sup>	73.33±7.64 <sup>d</sup>	1.53±0.36 <sup>b</sup>
Significance						
Accession		ns	ns	**	**	ns
Accession WA×Treatment		**	**	***	***	ns
Accession DA×Treatment		**	**	***	***	ns

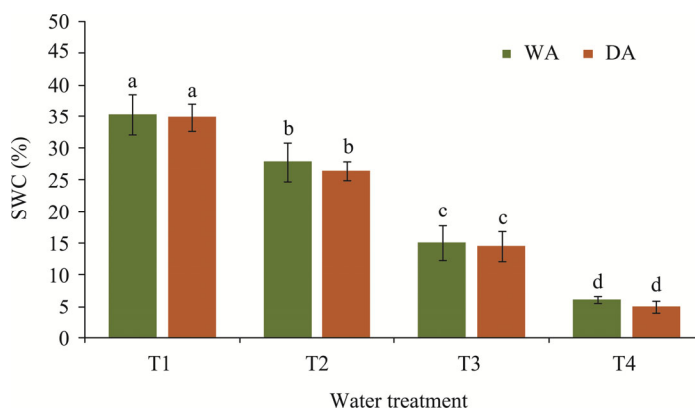
Note: ns, not significant; \*, significance at  $P<0.05$  level; \*\*, significance at  $P<0.01$  level; \*\*\*, significance at  $P<0.001$  level. Different lowercase letters in each column indicate significantly different at  $P<0.05$  level among the four water treatments for the same accession.

Throughout the experimental period, SWC was significantly lower under severe stress treatment (T<sub>4</sub>) than under other treatments (T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>). SWC differed significantly depending on the treatment (Fig. 2). The decrease of SWC generated a rapid reduction in  $\Psi_{pd}$  and  $\Psi_{md}$  under all treatments. Such significant differences among treatments are highlighted in Figure 2. In both accessions,  $\Psi_{pd}$  was significantly lower for very drought-treated *S. ciliata* plants than for drought-treated plants. Additionally,  $\Psi_s$  decreased significantly with the increase in water stress ( $P<0.01$ ; Fig. 3). The values of  $\Psi_{md}$  were very low under treatment T<sub>4</sub>, at -3.54 and -3.48 MPa, respectively, for accessions WA and DA. The correlations among  $\Psi_{pd}$ ,  $\Psi_{md}$  and  $\Psi_s$  under all treatments are illustrated in Table 2. The strongest correlation was found between  $\Psi_{md}$  and  $\Psi_{pd}$ , with  $R^2=0.966$ .

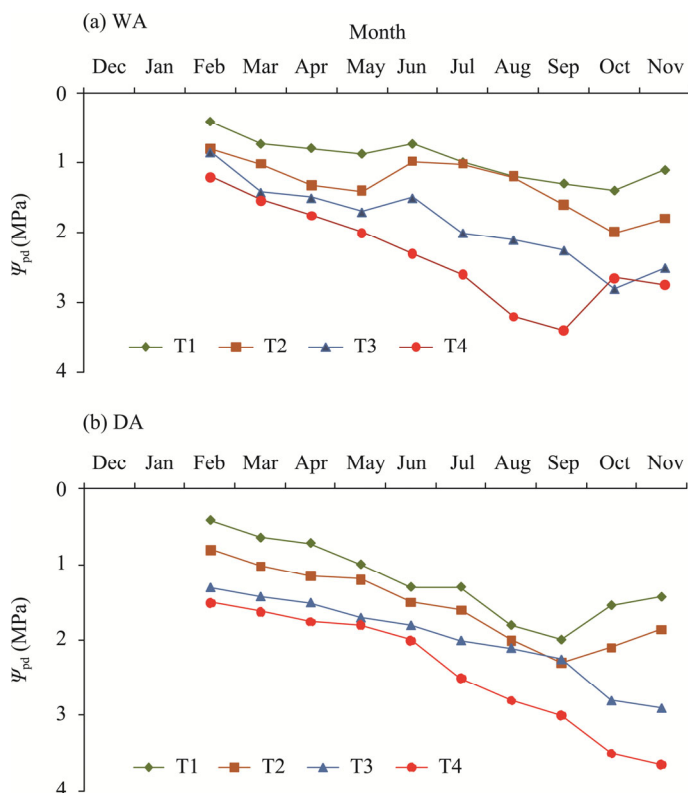
The decreases of  $P_n$  and  $g_s$  were closely correlated to the severity of water stress, exhibiting a high correlation with leaf water potential and SWC.  $P_n$  exhibited negative correlations with  $\Psi_{pd}$  and  $\Psi_{md}$  (Table 2).

### 3.3 Principal component analysis (PCA) biplot of phenological and physiological parameters

In the first axis of PCA (PC1) (79.24% of the total variance), a positive correlation was proved in total dry biomass, shoot dry biomass,  $P_n$ ,  $g_s$ , and tiller and leaf numbers (Fig. 4). In the second axis of PCA (PC2), which explained 14.66% of the total variance, root dry biomass,  $\Psi_{md}$ ,  $\Psi_{pd}$ , R/S ratio and  $\Psi_s$  associated positively, while these and other variables (spike number and SWC)



**Fig. 2** Soil water content (SWC) of two *S. ciliata* accessions under different water treatments. The water treatments (water stress levels) of plants were designed as follows: 200 mm/a for T<sub>1</sub>, 150 mm/a for T<sub>2</sub>, 100 mm/a for T<sub>3</sub> and 50 mm/a for T<sub>4</sub>. *S. ciliata* accession from a wet arid region (Bou Hedma National Park) was coded as WA, and *S. ciliata* accession from a dry arid region (Matmata Mountain) was coded as DA. Columns with the same lowercase letters are not significantly different between WA and DA, as determined by the Tukey's test ( $P < 0.05$ ).

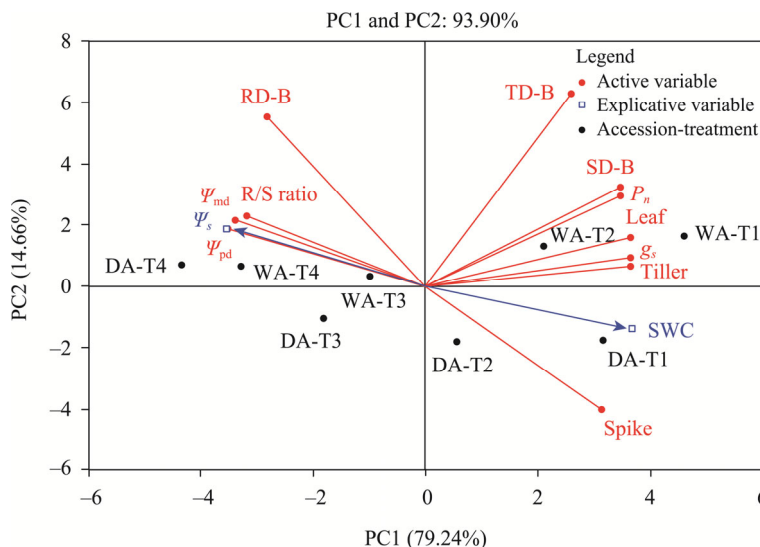


**Fig. 3** Predraw leaf water potential ( $\Psi_{pd}$ ) in *S. ciliata* accessions WA (a) and DA (b) under different water treatments (T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>)

associated negatively. The spike number was not associated with any variable. Dispersion of *S. ciliata* accessions on the principal components of PC1 and PC2 proved the different responses of both accessions to different water treatments. However, accession WA was detected in the positive extremity of PC1 and PC2. It was characterized by a large number of leaves and tillers and high shoot biomass production under treatments T<sub>1</sub> and T<sub>2</sub>. Accession DA was located in the negative extremity of PC1 and PC2 under treatments T<sub>1</sub> and T<sub>2</sub>. This accession was characterized by a high



number of spikes. Under treatments  $T_3$  and  $T_4$ , both accessions were detected in the negative extremity of the two axes (PC1 and PC2). They were characterized by a high root dry biomass. In this case, *S. ciliata* plants responded to water stress by producing more root biomass, closing their stomata and decreasing  $P_n$  and  $g_s$ . These results were confirmed by the highly negative correlations of physiological parameters ( $P_n$  and  $g_s$ ) with root dry biomass and SWC (Table 2).



**Fig. 4** Biplot of principal component analysis (PCA) showing the separation of phenological and physiological parameters of both *S. ciliata* accessions (WA and DA) under different water treatments. RD-B, root dry biomass; SD-B, shoot dry biomass; TD-B, total dry biomass;  $\Psi_{md}$ , midday leaf water potential;  $\Psi_{pd}$ , predawn leaf water potential;  $\Psi_s$ , soil water potential;  $P_n$ , net photosynthesis;  $g_s$ , stomatal conductance; SWC, soil water content. Red mark, active variable; blue mark, explicative variable; black mark, accession-treatment.

## 4 Discussion

Perennial grass phenological responses to water deficit have been extensively studied, with a special attention on the growth and biomass accumulation of plants (Anjum et al., 2011; Hamdani et al., 2019; Mnif Fakhfakh et al., 2020). The choice of highly tolerant grass species plays a crucial role in the restoration of degraded ecosystems. The selected plant species should have high drought tolerance to maintain ecosystem biodiversity. *S. ciliata*, as well as many other Mediterranean and perennial grasses, exhibits a conservative strategy in the use of water, based mainly on avoiding water stress (van Rooyen et al., 1991). Understanding the phenological and physiological responses of plants to water deficit is an important basis for selecting tolerant plant species. In this study, the results demonstrated that there were significant differences in the drought responses of two *S. ciliata* accessions coming from different arid regions of Tunisia. However, water stress had different effects on plant growth according to accession type.

Accession WA and accession DA highlighted different phenological adaptations by reducing leaf number, tiller number and shoot dry biomass. The growth reduction of *S. ciliata* accessions under different water stress conditions is similar to the responses of other perennial grasses like *Cenchrus ciliaris* (L.) and *Stipa tenacissima* (L.). For grasses, the tillering stage is an important parameter commonly used in the determination of the stress tolerance of plants (Fernandez and Reynolds, 2000). Notably, during the observations of the phenological parameters of *S. ciliata*, we found that leaf and tiller numbers decreased significantly with increasing water stress. In this study, tiller and leaf numbers varied significantly with water treatment and accession. Du et al. (2010) emphasized that the reduction of leaf growth is generally the primary response to water stress. These results were proved by the positive correlation between leaf and tiller numbers and

SWC (Table 2). In addition, leaf phenological plasticity is very crucial in terms of coping with water stress, because the first response of plant to water stress is the reduction of leaf growth. Guo et al. (2007) reported that the growth of grasses is greatly inhibited under drought conditions. Zhao et al. (2020) highlighted that *S. ciliata* persists well in desert habitats owing to the species' ability to respond with important growth to precipitation and its tolerance to drought and grazing.

In our study, severe drought stress treatments ( $T_3$  and  $T_4$ ) led to an increase in root dry biomass. However, Xiong et al. (2006) proved that under water stress conditions, the decrease of plant growth results in a reduction of shoot biomass and an increase of root biomass. The significant negative correlation between shoot dry biomass and root dry biomass mentioned in Table 2 showed that plants search for more water in the soil. However, the perennial grass (*S. ciliata*) increased the R/S ratio to overcome the water deficit, which increased water absorption and reduced water consumption. This response showed that under the severe water treatments  $T_3$  and  $T_4$ , both *S. ciliata* accessions (WA and DA) increased the belowground fraction and decreased the aboveground fraction. The significant increase in the R/S ratio ( $>1.29$ ) under severe water deficit treatment ( $T_4$ ) was the result of a significant decrease in shoot dry biomass and a significant increase in root dry biomass.

In this study, the shoot dry biomass and root dry biomass of *S. ciliata* varied considerably and significantly with the quantity of soil water. However, the shoot dry biomass of this perennial grass declined and root dry biomass concurrently increased with the increase in drought level, thus leading to a high R/S ratio. Henschel et al. (2005) asserted that the biomass production of *S. ciliata* varies considerably with the changes of precipitation. Indeed, roots are the first part exposed to water stress. Even though plant growth rates are decreased when soil water supply is limited, shoot growth tends to be slower than root growth. Chaieb et al. (1996) emphasized that the root system of *S. ciliata* has a high concentration of roots in the upper soil layer (0–30 cm depth). The increase in *S. ciliata* root biomass (between 20 and 100 cm) is in good conformity with those of certain studies carried out in arid regions (Chaieb et al., 1996). We can, therefore, conclude that the roots of plants in arid regions are a satisfactory solution to better utilize the soil water reserves. The root system can assure the production of plants species, although locally deep roots (30–80 cm layer) need to maintain not only the production but also species survival under water stress conditions, because at these depths, the soil is not completely dry. A reduction in water can reduce the number of spikes in both *S. ciliata* accessions (WA and DA). However, other studies have also proven the reduction of this parameter (spike number) describing the reproductive growth phase (Mnif Fakhfakh et al., 2018, 2020). The essential variations inferred for phenological parameters exhibited a wide phenological variability among *S. ciliata* accessions in response to different water treatments, reinforcing their adaptation to water stress resistance.

Soil water stress is a major environmental factor forbidding the productivity and photosynthesis of plants in arid regions. *S. ciliata* is renowned for its drought resistance (Mnif Fakhfakh et al., 2020). Nevertheless, there were no differences in drought resistance among both accessions (WA and DA) deriving from different bioclimate environments. Therefore, the loss of water resulted in the poor adaptation of plants to the water deficit. It has been shown that osmotic adjustment can be an intrinsic mechanism of drought resistance, operating by lowering the water potential at which stomatal closure appear when the stomatal control of transpiration is not adequate for regulating water supply to leaf cells (Farman et al., 2017). This may sometimes be accounted for in terms of interpreted conferring the inherent resistance to the water stress of  $C_4$  plants (Sage, 2004). This goes in good accordance with the results obtained for both studied accessions of *S. ciliata* in the current study. The photosynthetic pathway of  $C_4$  plants has developed as an adaptation to elevated photo-respiratory pressures resulting from the combinations of stress, which include low  $CO_2$  concentration, high temperature, aridity and salinity (Sage, 2004). Yu al. (2009) proved that  $C_4$  plants present various adaptation strategies to drought, to minimize the damage from environmental stress. Indeed, leaf gas exchange measurements confirmed the significant differences in  $CO_2$  assimilation and  $g_s$  characteristics among seedlings under water

deficit conditions. Moreover, plants can adapt to water deficit through the modification of phenological and physiological characteristics (Zhao et al., 2020). From this perspective, in this study, the phenological and physiological variations of *S. ciliata* plants were remarkable under water stress conditions (Fig. 4).

Generally, it has been proven that the dry soil has a hydraulic effect on plant water status and leaf gas exchange. The basic theory is that a decrease in  $\Psi_s$  requires a reduction in plant water potential, inducing reduced cell expansion and stomatal closure. This is confirmed in this study by the strong correlation between  $P_n$  and  $\Psi_{pd}$  of both *S. ciliata* accessions (Table 2). Stomatal closure as well as leaf expansion is controlled by mechanisms additional to changes in the pressure potential of leaf cells (Kadioglu and Terzi, 2007). Reductions in  $g_s$  of plants in the dry soil are related more strongly to changes in soil water status rather than leaf water status. It has been noted that plants can 'sense' that the soil in the root zone is drying and can communicate this information to the leaves by another means than through a decrease in leaf water status (Kellogg, 2013). This is consistent with our results, which yielded a significant difference in  $g_s$  for all water treatments. The effect of water stress on  $P_n$  has been little explored, and the decreases in  $P_n$  and  $g_s$  were observed with increasing water stress. However, this reduction has been attributed to both non-stomatal and stomatal limitations. Kadioglu and Terzi (2007) reported that  $g_s$  was proved to be a reliable physiological indicator of drought tolerance. As far as our research is concerned, it was found that both accessions WA and DA presented gradual decreases in  $P_n$  and  $g_s$  with decreasing  $\Psi_{pd}$  and  $\Psi_s$ , confirming this important relationship between  $\Psi_{pd}$  and  $P_n$  under severe drought condition (treatment T<sub>4</sub>). Furthermore, Yu et al. (2009) pointed out that stomatal closure is the first line of defence against desiccation for plants. Sayed (2003) revealed that water stress-induced stomatal closure depletes intercellular CO<sub>2</sub>, leading to photoinhibition. Stomatal control is crucial for adjusting leaf gas exchange, especially for plants in arid regions (Yu et al., 2009). Stomatal closure and leaf growth inhibition are among the primary plant responses to drought, preserving the plants from intense water loss.

Both phenological and physiological changes exist when plants are subjected to water deficit (Xu et al., 2006; Gazanchian et al., 2007). Indeed, water stress may reduce  $P_n$  by stomatal closure and metabolic processes. Liu et al. (2006) proved that physiological parameters are more responsive than phenological parameters under different drought conditions. The data of this study corroborated that for this C<sub>4</sub> species (*S. ciliata*), SWC affected the leaf water potential. Thus, in case of water stress, this grass species with fasciculate and superficial root systems tend to adjust their water transfer by stopping their leaf transpiration. This observation was similar with other C<sub>4</sub> grasses (*C. ciliaris*, *Stipa lagascae* R. & Sch. and *Digitaria nodosa* Parl.) of the same region (Chaieb et al., 1996). Under arid and semi-arid bioclimate conditions, soil drought was reported to impact plants from several aspects (Du et al., 2010). Roots are the first part of the plants suffering from water stress. Plants grow slowly with limited SWC due to the decrease in photosynthesis, resulting in reduced biomass (Anjum et al., 2011). Leaf water potential also decreases during the periods of drought. Plants take up water from the soil and transpire it through the leaves. Leaf water potential decreases with the reduction in SWC as the rate of leaf water loss exceeds the rate of root water uptake.

## 5 Conclusions

The knowledge of plant growth under water deficit is crucial in terms of assessing the productivity of rangelands and grasslands. In this regard, the hypothesis postulating that *S. ciliata* accessions present a high capacity to tolerate drought imposed by water stress was confirmed. Accession WA (coming from the Bou Hedma National Park) was more resistant than accession DA (coming from the Matmata Mountain). The ability of *S. ciliata* to keep photosynthetic active tissue has proved that the species has a higher rooting rate, with a tendency to extract the soil water. These strategies ensure that plants are well regulated in gas exchanges under arid

conditions. Water stress seems to be a crucial factor limiting the  $P_n$  process of *S. ciliata* accessions. These results are indicative that this perennial grass adopts the strategy of avoidance (stomatal closure) as a drought resistance mechanism that may probably prevent physiological and biochemical damages to plants during the drought periods. It is noteworthy that a close relationship between SWC and plant growth and physiological processes was recorded for both accessions of *S. ciliata*. At this stage of analysis, this behaviour, therefore, stands for an endogenous characteristic of this pastoral species, which is a potential candidate for the ecological restoration of degraded rangelands and grasslands in North Africa.

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